REMARKS

The present response is intended to be fully responsive to all points of objection and/or rejection raised by the Examiner and is believed to place the application in condition for allowance. Favorable reconsideration and allowance of the application is respectfully requested.

Applicants assert that the present invention is new, non-obvious and useful. Prompt consideration and allowance of the claims is respectfully requested.

Status of Claims

Claims 1, 2, 8, 10-18, 20, 22-31, 43, 46-48, 63 and 66-71 are pending. Claims 3-5, 7, 9, 19, 21, 32-38, 40, 42, 44, 49-62, 64 and 65 have been withdrawn. Claims 6 and 45 have been cancelled without prejudice or disclaimer. In making this cancellation without prejudice, Applicants reserve all rights in these claims to file divisional and/or continuation patent applications.

Double Patenting Rejections

In the Office Action, the Examiner provisionally rejected claims 1, 2, 8, 10, 14 and 16 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 16-26 of copending Application no. 10/473,180.

Applicants will provide a terminal disclaimer upon indication by the Examiner of allowable claims.

In the Office Action, the Examiner provisionally rejected claims 1, 2, 8, 10-13, 18 ,20, 22-24 and 26-30 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 13, 18, 19, 24, 29 and 30 of copending Application no. 11/204,391.

Applicants will provide a terminal disclaimer upon indication by the Examiner of allowable claims.

In the Office Action, the Examiner rejected claims 1, 2, 6, 8, 10-18, 20 and 22-31 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 6, 16, 17, 19 and 20 of US Patent No. 7,655,445.

Applicants will provide a terminal disclaimer upon indication by the Examiner of allowable claims.

CLAIM REJECTIONS

35 U.S.C. §103 Rejections

The Examiner has rejected claims 1, 2, 8, 10-18, 20, 22-31, 46, 47 and 67-70 under 35 U.S.C. §103(a), as being unpatentable over Pikas (IDS dated 26 December 2006), in view of Habuchi *et al.* (of record), in view of Koeller (PTO-892, Ref. V), in view of Toone *et al.* (PTO-892, Ref.W), in view of Petterson et al (PTO-892, Ref. U) and further in view of Kobayashi et al (PTO-892, Ref. V).

Claim 1 is directed to a method of preparing a sulfated polysaccharide capable of binding to a protein, consisting of stepwise enzymatically treating an unsulfated or incompletely sulfated polysaccharide with a mixture of enzymes, said mixture of enzymes consisting of *N*-deacetylase-*N*-sulfotransferase (NDST), optionally heparitinase; optionally an epimerase, at least one *O*- sulfotransferase selected from the group consisting of 2-OST, 6-OST, 3-OST; optionally $\Delta^{4,5}$ glycuronidase; wherein the order of the enzymatic steps can be varied and wherein said method comprises at least one in vitro step.

Claim 2 is directed to a method of preparing heparan sulfate, consisting of stepwise enzymatically treating an unsulfated heparan synthon or incompletely-sulfated heparan sulfate precursor with a mixture of enzymes, said mixture of enzymes consisting of N-deacetylase-*N*-sulfotransferase (NDST);optionally heparitinase;optionally an epimerase, at least one *O*- sulfotransferase is selected from the group consisting of 2-OST, 6-OST, 3-OST, optionally $\Delta^{4,5}$ glycuronidase, and wherein the order of the enzymatic steps can be varied and wherein said method comprises at least one in vitro step.

The Examiner is stating that it would have been obvious for one of ordinary skill in the art to combine the teachings of Pikas concerning the modification of a polysaccharide having the structure (GlcA ß1-4GlcNAcά1-4)$_n$ by chemical N-deacetylation and N-sulfation, enzymatic GlcA C5-epimerization and chemical O-sulfation, with the teachings of Habuchi reagarding the availability and substrate specificity of the different enzymes involved in the biosynthesis of heparin sulfate with the teachings of Koeller regarding the use of carbohydrate enzymes as a tool for the synthesis of complex carbohydrates, with the teachings of Toone regarding enzymes as catalysts in carbohydrate synthesis, with the teachings of Petterson regarding the purification of a mouse mastocytoma protein required for glucosaminyl N-deacetylation and N-sulfation, with the teachings of Kobayashi regarding the purification and characterization of heparin sulfate 2-sulfotransferase from CHO cells.

However, Pikas teaches a sulfation process that is primarily chemical Habuchi teaches the *in vivo* activity of the various enzymes. Koeller refers only to the enzymatic synthesis of an unsulfated or incompletely sulfated polysaccharide using glycosyltransferases (page 1158) and does not refer to epimerases or O-sulfotransferases. Toone *et al.* teaches the enzymatic synthesis of saccharide monomers and of some saccharide oligomers (schemes 2-20) and does not refer to epimerases or O-sulfotransferases. Petterson teaches the purification of a mouse mastocloma protein required for glucosaminyl N-deacetylation and N-sulfation. Kobayashi teaches the purification and characterization of heparin sulfate 2-sulfotransferase from cultured CHO cells.

As stated by the Examiner, the teachings of Pikas differ from the instantly claimed invention in that O-sulfation of the polysaccharide was accomplished chemically rather than enzymatically. Koeller and Toone do not describe the activities of epimerases and of O-sulfotransferases and since Habuchi and Pikas (and Koeller and Toone) do not teach the stepwise enzymatic *in vitro* synthesis of sulfated polysaccharides and a stepwise, in-vitro, enzymatic process such as the method disclosed claims 1 and 2 is not obvious in view of a corresponding *in-vivo* process. This is because *in vitro* synthetic parameters such as temperature, pH, reaction times, concentrations, atmosphere, and ingredients must be carefully selected in order for each step to work.

Furthermore, neither Kobayashi nor Petterson use a size defined substrates to study the action of enzymes. It is known in the art that the action of enzymes are greatly influenced by the size as much of the precursor structures with defined modifications. Kobayashi and Petterson use mainly heparin/heparan sulfate of undefined composition or structures and the results do not reveal the nature of action in terms of extent of action, the site of action adjacent in the oligosaccharides. By contrast, Applicants' invention makes use of a hexasaccharide to generate a defined anticoagulant structure whose sequence was deduced with mass spectrometry. Different isoforms of 6-OST were used in different combination to show that a minimum of two isoforms are required to the biologically active domain. Applicants have also shown that C5-epimerase needs to be coupled with 2OST enzyme to produce the binding domain at the hexasaccharide level. None of the references cited have used these enzymes together and a person skilled in the art would not use the teachings of the references to come up with Applicants' invention that uses more than one enzyme together and produces an oligosachcaride product that can bind to ATIII.

Applicants assert that Pikas in view of Habuchi, Koeller, Toone, Petterson and Kobayashi cited alone or in combination do not teach or suggest a "method of preparing a sulfated polysaccharide capable of binding to a protein, comprising stepwise treating an unsulfated or incompletely sulfated polysaccharide with enzymes, wherein said enzymes consists of: N-deacetylase-N-sulfotransferase (NDST), optionally heparitinase, optionally C-5 epimerase, at least one OST selected form a group consisting of 2-OST, 6-OST, 3-OST, optionally $\Delta^{4,5}$ glycuronidase, wherein the order of the enzymatic steps can be varied and wherein said method comprises at least one in vitro step" (Emphasis added). Furthermore, Habuchi, Koeller, Toone, Petterson and Kobayashi do not remedy the deficiencies of Pikas, and Pikas in view of Habuchi, Koeller, Toone, Petterson and Kobayashi do not render obvious Applicants' invention as recited in claims 1 and 2 and claims 11, 12, 18, 20, 22-31, 47, 47, 69 and 70 which depend from and/or incorporate the limitations of claims 1 and 2. Therefore, Applicants respectfully request withdrawal of the rejection.

The Examiner has rejected claims 43 and 48 under 35 U.S.C. §103(a), as being unpatentable over Pikas (IDS dated 26 December 2006) and further in view of Habuchi (of record), in view of Koeller (PTO-892, Ref. V), in view of Toone (PTO-892, Ref. W), in view

of Pettersson (PTO-892, ref. U), in view of Kobayashi (PTO-892, Ref. V), as applied to claims 1, 2, 8, 10-18, 20, 22-31, 46, 47 and 67-70, and further in view of van Boeckel (PTO-892, Ref. X), in view of Kusche (of record), in view of Nader (of record) and in view of Myette (of record).

Claims 43 and 48 depend from and incorporate the limitations of claim 1.

As discussed above, claim 1 is not rendered obvious over Pikas in view of Habuchi, Koeller, Toone, Petterson and Kobayashi and claims 43 and 48 which depend from and incorporate the limitations of claim 1 are therefore not rendered obvious by Pikas in view of Habuchi, Koeller, Toone, Petterson and Kobayashi.

The Examiner is stating that van Boeckel teaches the antithrombin III binding domain of heparin is a lead to new synthetic antithrombotics. Since the ability of heparin fragments to reinforce ATIII-mediation inhibition of factor Xa appeared independent of their size, it was logical to look for the smallest fragments able to catalyze inhibition of factor Xa. The Examiner is stating that since van Boeckel teaches that no biochemical tool for further controlled degradation was available at the time, chemical synthesis was required. Furthermore, van Boeckel does not teach nor disclose enzymatic synthesis using the combination of specific isoforms and sizes to generate ATIII binding sequences/structures. Van Boeckel teaches targets that are different than the target disclosed in Applicants' invention. The reducing end lacks 6-O sulfate groups on the hexoamine residues in the pentasachcaride. Furthermore, it has N-acetyl group on the non-reducing end. Van Boeckel does not teach nor disclose the use of pentasaccharide that was generated using intermediates such as anydro-forms and reactive, less stable, light sensitive azido sugars, etc. Van Boekel teaches the use of light sensitive, water-reactive intermediates such as azido sugar, anhydrosugars, reactive unstable species. By contrast, Applicants' invention makes use of intermediates which are stable to light, water, etc. Therefore, van Boeckel does not render obvious Applicants' invention.

Kusche teaches the biosynthesis of heparin and the various substituents of heparin that are important for antithrombin binding. However, Kusche teaches the use of pentasaccharide but failed to teach the use of the specific enzymes and indicate which enzyme acts on which site within the pentasaccharide. Kusche reaches the use of macrosomal fractions that contain

many of the enzymes and Kusche obtained only 0.5% of the total product to have high affinity for binding to ATIII (See, page 7400, right column). Kusche does not teach nor disclose all the enzymes necessary in the generation of the ATIII binding domain. Applicants' invention showed greater than 80% bound to ATIII indicating that specific structures were generated. Kusche does not teach nor disclose the incorporation of 3-O sulfate group, an essential feature for binding to ATIII and Kusche teaches that the oligosaccharides did not to incorporate 3-O sulfate groups (See page 7400, right column). Therefore, Kusche does not render obvious Applicants' invention.

Nader teaches the purification and substrate specificity of heparitinase I and heparitinase II. Nader teaches the cleavage of the heparan sulfate/heparin structure rather than the assembly of ATIII binding motif and the heparin lyases were used to examine the disaccharide profiles. Nader does not teach nor disclose the generation of oligosaccharides. By contrast, Applicants' invention is directed to the generation of specific, size-defined oligosaccharides that were subjected to enzymatic modification to synthesize/construct ATIII binding domains. Therefore, Nader does not render obvious Applicants' invention.

Myette teaches the cloning and substrate specificity of the heparin/heparin sulfate which discriminate both on the basis of glycosidic linkage and sulfation pattern. However, Myette fails to demonstrate that the Δ-4,5-glycouronidase could act on the oligosaccharide structure that we used and the teachings of Myette are limited to the use of disaccharides that do not bind to ATIII. By contrast, Applicants' invention demonstrates the action of the enzyme and the generation of unique heparin/heparan sulfate oligosaccharide structure using recombinant enzymes. Therefore, Myetter does not render obvious Applicants' invention.

Therefore, van Boeckel, Kushe, Nader and Myette do not cure the deficiencies of Pikas in view of Habuchi, Koeller, Toone, Petterson and Pikas in view of Habuchi, Koeller, Toone, van Boeckel, Kusche, Nader and Myette do not render obvious Applicants' claimed invention. Accordingly, Applicants respectfully request withdrawal of the rejection.

The Examiner has rejected claims 63, 66 and 71 under 35 U.S.C. §103(a), as being unpatentable over van Boeckel *et al.* (PTO-892, Ref. X), in view of Kusche *et al.* (of record), in view of Pikas (IDS dated 26 December 2006) in view of Habuchi *et al.* (PTO-892, of record), in view of Nader *et al.* (of record), in view of Myette *et al.* (of record), in view of Koeller *et al.* (PTO-892, Ref. V) and in view of Toone *et al.* (PTO-892, Ref. W).

Claim 63 is directed to an in vivo enzymatic method of producing pentasaccharaide 15. Claim 66 is directed to an enzymatic method of synthesizing pentasaccharide 15 wherein said method comprises at least one in vitro step.

Claim 71 depends from and incorporate the limitations of claim 66.

The Examiner is stating that the teachings of van Boeckel differ from the instantly claimed invention in that van Boeckel does not disclose the synthesis of the ATIII-binding pentasaccharide by enzymatic methods. Furthermore, van Boeckel does not teach nor disclose enzymatic synthesis using the combination of specific isoforms and sizes to generate ATIII binding sequences/structures. Van Boeckel teaches targets that are different than the target disclosed in Applicants' invention. The reducing end lacks 6-O sulfate groups on the hexoamine residues in the pentasachcaride. Furthermore, it has N-acetyl group on the non-reducing end. Therefore, van Boeckel does not render obvious Applicants' invention.

Kusche teaches the biosynthesis of heparin and the various substituents of heparin that are important for antithrombin binding. However, Kusche teaches the use of pentasaccharide but failed to teach the use of the specific enzymes and indicate which enzyme acts on which site within the pentasaccharide. Kusche reaches the use of macrosomal fractions that contain many of the enzymes and Kusche obtained only 0.5% of the total product to have high affinity for binding to ATIII (See, page 7400, right column). Kusche does not teach nor disclose all the enzymes necessary in the generation of the ATIII binding domain. Applicants' invention showed greater than 80% bound to ATIII indicating that specific structures were generated. Kusche does not teach nor disclose the incorporation of 3-O sulfate group, an essential feature for binding to ATIII and Kusche teaches that the oligosaccharides did not to incorporate 3-O sulfate groups (See page 7400, right column). Therefore, Kusche does not render obvious Applicants' invention.

Pikas teaches a sulfation process that is primarily chemical Habuchi teaches the *in vivo* activity of the various enzymes. Koeller refers only to the enzymatic synthesis of an unsulfated or incompletely sulfated polysaccharide using glycosyltransferases (page 1158) and does not refer to epimerases or O-sulfotransferases. Toone *et al.* teaches the enzymatic synthesis of saccharide monomers and of some saccharide oligomers (schemes 2-20) and does not refer to epimerases or O-sulfotransferases. As stated by the Examiner, the teachings

of Pikas differ from the instantly claimed invention in that O-sulfation of the polysaccharide was accomplished chemically rather than enzymatically.

Koeller and Toone do not describe the activities of epimerases and of O-sulfotransferases and since Habuchi and Pikas (and Koeller and Toone) do not teach the stepwise enzymatic *in vitro* synthesis of sulfated polysaccharides and a stepwise, in-vitro, enzymatic process such as the method disclosed claims 1 and 2 is not obvious in view of a corresponding *in-vivo* process. This is because *in vitro* synthetic parameters such as temperature, pH, reaction times, concentrations, atmosphere, and ingredients must be carefully selected in order for each step to work. Therefore, Pikas, Habuchi, Keoller and Toone do not render obvious Applicants' invention.

Nader teaches the purification and substrate specificity of heparitinase I and heparitinase II. Nader teaches the cleavage of the heparan sulfate/heparin structure rather than the assembly of ATIII binding motif and the heparin lyases were used to examine the disaccharide profiles. Nader does not teach nor disclose the generation of oligosaccharides. By contrast, Applicants' invention is directed to the generation of specific, size-defined oligosaccharides that were subjected to enzymatic modification to synthesize/construct ATIII binding domains. Therefore, Nader does not render obvious Applicants' invention.

Myette teaches the cloning and substrate specificity of the heparin/heparin sulfate which discriminate both on the basis of glycosidic linkage and sulfation pattern. However, Myette fails to demonstrate that the Δ-4,5-glycouronidase could act on the oligosaccharide structure that we used and the teachings of Myette are limited to the use of disaccharides that do not bind to ATIII. By contrast, Applicants' invention demonstrates the action of the enzyme and the generation of unique heparin/heparan sulfate oligosaccharide structure using recombinant enzymes. Therefore, Myetter does not render obvious Applicants' invention.

Therefore, van Boeckel, in view of Kusche, in view of Pikas, in view of Habuchi, in view of Nader, in view of Myette, in view of Koeller and in view of Toone do not disclose a stepwise, enzymatic *in vitro* sulfation process that consists of the epimerases and *O*-sulfotransferases as does the present invention. Therefore, van Boeckel *et al.* in view of Kusche *et al.*, Pikas, Habuchi *et al.*, Nader *et al.*, Myette *et al.*, Koeller *et al.* and Toone *et al.*

do not render obvious Applicants claimed invention. Accordingly, Applicants respectfully request withdrawal of the rejection.

Conclusion

In view of the foregoing amendments and remarks, Applicants assert that the pending claims are allowable. Their favorable reconsideration and allowance is respectfully requested.

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this paper to deposit account No. 50-3355.

Respectfully submitted,

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